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土壤微藻对盐胁迫的响应及其 对盐渍化土壤的改良作用

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摘 要:土壤盐渍化已成为世界性环境问题.为探究盐渍化土壤生物改良新技术,利用从新疆盐渍化土壤表层生物结皮中筛选的一株微藻,通过室内模拟实验,研究了该藻的耐盐性能及其对盐碱土的改良作用.结果表明:该藻具有较高的耐盐性,能够在1 mol/L NaCl溶液中存活并生长;盐胁迫降低了微藻光合色素的含量,表现出明显的梯度效应;经过18 d的培养,0.5 mol/L,1.0 mol/L,1.5 mol/L NaCl处理组中可溶性盐分别下降16.99%,9.23%,3.27%;将土壤微藻接种在高盐碱土表层,初始叶绿素 a 为 3 μg/cm²,5 μg/cm²,8 μg/cm² 土壤的实验组,经过20 d 培育后,土壤含水率分别增加了29.41%,38.29%,39.54%,胞外聚合物(EPS)分别增加了82.84%,86.04%,116.06%.说明土壤微藻具有降低可溶性盐分,控制土壤盐分运移,以及保持土壤水分的作用.该研究为土壤藻改良盐渍化土壤提供重要的理论依据.

关键词: 土壤藻; 盐胁迫; 盐碱土; 土壤改良; 含水率; 环境地质.

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Response of Soil Microalgae to Salt Stress and Its Improvement Effect on Salinized Soil

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Abstract: Soil salinization has become a worldwide environmental problem. In order to explore the new biological technology for

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salinized soil improvement, laboratory simulation experiments were carried out in this study, in which an indigenous microalgae isolated from the biological soil crust of salinized soil in Xinjiang was used to explore the salt tolerance of the algae and its effect on saline-alkali soil. The results show that the microalgae had high salt tolerance to grow in 1 mol/L NaCl solution. The synthesis of algal photosynthetic pigments was inhibited by salt stress with obvious gradient effect. After 18 d incubation, the contents of the soluble salts in 0.5 mol/L, 1.0 mol/L, 1.5 mol/L NaCl treatment groups decreased by 16.99%, 9.23%, and 3.27%, respectively. The soil microalgae were inoculated and cultivated on the surface of high saline-alkali soil. The experimental groups with initial chlorophyll a of 3 μ g/cm², 5 μ g/cm² and 8 μ g/cm² soil, the soil moisture content increased by 29.41%, 38.29% and 39.54%, and extracellular polymers (EPS) increased by 82.84%, 86.04%, and 116.06%, respectively. These results indicate that soil microalgae can reduce soluble salt, control soil salt transport, and maintain soil moisture. This study provides an important theoretical basis for saline soil improvement by soil microalgae.

Key words: soil algae; salt stress; saline alkali soil; soil improvement; water content; environmental geology.

随着全球土地资源逐年减少,盐碱地作为重要的土地资源,其综合治理与开发利用越来越受到人们的关注和重视.土壤盐渍化是世界性环境问题之一,已经影响了全球6%的陆地面积(Nisha et al., 2018),主要分布在全球干早半干旱地区.我国北方地区受生态环境和人为活动的影响,土壤盐渍化较严重(Wang et al., 2021a;陶彦臻等,2021;曾邯斌等,2021).

土壤藻作为初级生产者,是表层土壤微生物群落的重要组成部分(Jassey et al., 2022).土壤藻分泌胞外聚合物(EPS)可以吸附和螯合重金属离子和砷(Wang et al., 2021b; 钟兆淇等,2022).同样,藻类产生的胞外聚合物能够螯合土壤中的盐离子降低盐分含量(Gr et al., 2021).土壤藻在土壤表层生长,与土壤颗粒胶黏形成生物土壤结皮(Tiwari et al., 2019).生物土壤结皮影响土壤理化特性和水文过程(Kakeh et al., 2018; Chamizo et al., 2013).土壤藻通过光合作用和固氮作用,提高土壤肥力(Yandigeri et al., 2011; Singh et al., 2019; Alvarez et al., 2021),为后续植物的生长发育提供有利环境条件.

本研究从新疆塔里木河下游盐渍化土壤中分离纯化出一株微藻.利用液相实验,分析微藻对不同浓度 NaCl的耐盐性.通过向盐碱土表面接种微藻,培植土壤藻结皮,研究土壤藻对盐渍化土壤的生物改良作用.研究结果有助于为盐渍化土壤的生物修复技术提供理论依据.

1 材料与方法

1.1 土壤微藻的筛选与培养

从塔里木河下游盐渍化土壤表面采集土壤藻结皮,将10g新鲜藻结皮置于1000 mL无菌三角锥

形瓶中,并加入400 mL已灭菌的BG11培养基(Feng et al., 2011),在4000 Lx,28℃条件下无菌通气培养3d.将5~20 μL藻悬液均匀涂布在BG11固体培养基上,置于光照培养箱中相同条件持续光照培养15d,从BG11固体培养基平板上获取相同藻落并接种于100 mL已灭菌的BG11培养基中.经过2次稀释、重新涂布于BG11固体培养基上,挑选单藻落接种到无菌BG11培养基中以获得单藻落的纯培养物.采用显微镜镜检显示为一株球形微藻.

1.2 盐胁迫对土壤微藻生长的影响

将已纯化的微藻培养至对数生长期,经过离心、清洗,收集藻细胞.在500 mL无菌三角锥形瓶中,采用 NaCl(优级纯,国药集团)分别配置200 mL 盐度为 0 mol/L、0.5 mol/L、1 mol/L 和1.5 mol/L 的培养液.土壤藻的生物量用680 nm处的光密度 OD650 表示(Prajapati et al., 2014),用紫外可见分光光度计测定.采用 Lichtenthaler (1987)的方法提取和测定微藻叶绿素 a 和类胡萝卜素.初始藻浓度 OD650 为 0.2,持续培养18 d,定期采样测定.所有处理均在光照培养箱中培养,温度为 28 ± 1 °C,明暗循环为 12 h; 12 h,光照强度为 4 000 Lx,每天定期摇瓶 3 次.

1.3 土壤藻结皮培育

供试土壤样品采自新疆塔里木河下游盐碱地农田表层 0~10 cm 土壤.供试土壤 pH 为 9.37,含水率 为 12.5%, 电导率 为 3.11 ms/cm, 全 盐 量 为 17.26 g/kg. 土壤样品在室内自然风干,研磨后过 1 mm 筛网去除枯枝落叶及大颗粒杂质后备用.将60 g处理后的土壤样品装入直径 9 cm、高 2 cm 的玻璃培养皿中,土壤容重为 1.01 g/cm³.5 000 rmp 下离心 15 min 收集对数生长期的土壤藻,用无菌去离子水清洗 3次后,重悬于无菌去离子水中,将藻悬液均匀接种在土壤表面,使接种量(叶绿素 a)分别为

3 μg/cm²、5 μg/cm²、8 μg/cm²土壤. 对照组土壤表面均匀添加等量去离子水. 所有实验组均在 28±1℃,明暗循环为12 h:12 h,光照强度为4 000 Lx的光照培养箱中进行. 每隔 3 d 补充等量水分.

1.4 土壤理化性质测定

土壤 pH、EC 的测定参照 Zhan and Sun (2012)的方法.可溶性盐含量采用烘干法测定.表层结皮色素含量采用乙醇提取-紫外分光光度法测定(Castle et al., 2011).土壤 EPS采用硫酸-苯酚法测定(Lan et al., 2017).

1.5 数据分析

数据采用平均值 ± 标准差表示.使用 Origin 2018 软件,采用独立样本 T 检验进行显著性分析.采用皮尔逊相关系数检验来评价方差之间的关系.

2 结果与讨论

2.1 盐胁迫对土壤微藻生长的影响

微藻溶液的 OD 值变化在一定程度上能够反映微藻的生长. 如图 1 所示,添加不同浓度 NaCl处理组的小球藻生长均受到一定程度抑制,且盐浓度越高,抑制程度越大. 其中,1.5 mol/L NaCl处理组的微藻 OD 值在第 2 d 开始下降,表明小球藻受到盐胁迫出现死亡现象,第 4 d 降至最低,随后稳定在 0.15,表明微藻全部死亡. 而在 0.5 mol/L 和 1 mol/L NaCl处理组中,小球藻均能稳定增长,第 18 d 时 OD680 值分别为 2.43、1.31,表明微藻能够在该盐度条件下存活.

盐胁迫条件下,细胞分裂减缓,细胞体积减小,细胞运动缓慢(Shetty et al., 2019).藻细胞浓度随

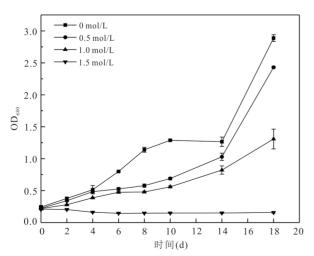


图1 盐胁迫下土壤微藻的生长

Fig. 1 Growth of soil microalgae under salt stress

盐浓度的增高而降低,主要原因是受到不同盐分的胁迫作用,导致细胞分裂减缓,细胞死亡,从而引起细胞密度降低.高盐胁迫下,能够破坏细胞生理过程,特别是光合作用,导致细胞生长受阻(Solovchenko et al., 2012).因此,1.5 mol/L NaCl处理组中微藻细胞生长明显受阻,第4d细胞全部死亡.随着微藻对盐胁迫的适应,细胞内蔗糖磷酸合成酶(SPS)被激活,合成并积累蔗糖等小分子化合物来抵抗逆境(Liang et al., 2020).因此,第10d后,0.5 mol/L 和1 mol/L 处理组的藻类生长速度持续增加.在盐度较高时略有阻碍,但它们的生长并没有停止(图1),这证实了该藻的长期耐盐性.

2.2 盐胁迫对土壤微藻色素的影响

图 2显示盐胁迫下土壤微藻色素含量变化.图 2a 表明,初始叶绿素 a 浓度约为 1 000 μg/L, 1.5 mol/L 盐度处理组在第 4 d 后接近 0,其余 3 组叶绿素 a 含量均稳定增长,说明高盐条件下,对叶绿素 a 的影响较大.0.5 mol/L和 1 mol/L 盐度处理组在前 10 d增长缓慢,分别增长了0.95×10³ μg/L、0.061×10³ μg/L,随后快速增长.其中 0.5 mol/L处理组在第 18 d 叶绿素 a 含量超过了对照组,含量为 11.4×10³ μg/L.由图 2b 可知,初始类胡萝卜含量约为 0.32×10³ μg/L,0.5 mol/L 处理组类胡萝卜素含量在前 10 d 缓慢增长,随后快速增长,第 18 d 含量为 2.56×10³ μg/L,而 1 mol/L 处理组类胡萝卜素含量增长较慢,第 18 d 含量为 0.83×10³ μg/L,仅增长了 0.57×10³ μg/L,说明较高浓度的盐胁迫对微藻类胡萝卜素有很强的抑制作用.

光合色素含量是反映植物光合作用能力的重要指标(Ji et al., 2018). 叶绿素 a 是光合作用必需的重要色素,其含量的高低间接反映微藻的光合效率. 类胡萝卜素吸收光能,是内源性抗氧化剂. 因此,叶绿素 a 和类胡萝卜素的含量反映了植物不同时期的生长发育状况. 叶绿素 a 和类胡萝卜素的变化整体上具有一致性. 但叶绿素 a 的损伤效果强于类胡萝卜素,其原因可能是叶绿素 a 的含量相对较高. 此外,类胡萝卜素提高细胞的抗氧化能力,有助于减少氧化损伤(Peng et al., 2021).

2.3 盐胁迫对土壤藻培养液中 pH 和 EC 的影响

图 3 为盐胁迫条件下微藻对培养基中 pH和EC 变化的影响.图 3a表明,在前 4 d,除 1.5 mol/L 盐度处理组外,其他处理组 pH均迅速上升.其中,对照组 pH值初始为 7.64,第 4 d迅速上升到 10.62,之后

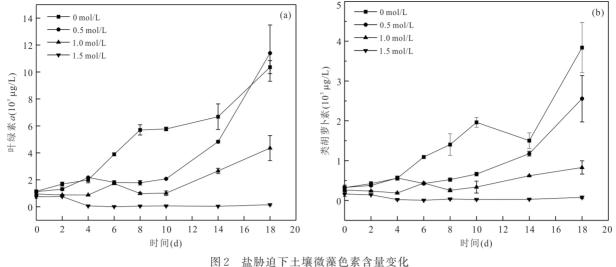


Fig. 2 Changes of soil microalgae pigment content under salt stress

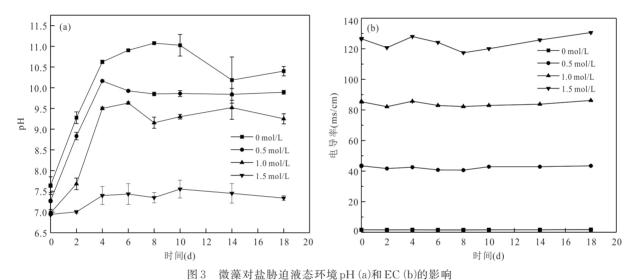


Fig. 3 Effects of microalgae on pH (a) and EC (b) in liquid environment under salt stress

趋于稳定;同样,0.5 mol/L 处理组 pH 值由 7.26 上升至 9.80, 最终稳定在 10; 1 mol/L 处理组 pH 值由 6.98 上升并稳定于 9.3; 1.5 mol/L 处理 组 pH 由 6.9 上升为 7.3,4 d 后趋于稳定.由此 可知,溶液盐度越高,最终稳定的pH值越低.

由图 3b 可知, 电导率整体呈波动趋势. 其中前 8d各处理组电导率总体呈下降趋势,对照组变化不 明显.第8d各实验组的电导率达到最低,其中, 0 mol/L 对照组由初始 1.63 ms/cm 下降到 1.53 ms/ cm, 0.5 mol/L、1 mol/L和1.5 mol/L处理组分别由 43.43 ms/cm、85.40 ms/cm、126.53 ms/cm 下降为 40.63 ms/cm、82.2 ms/cm 和 117.43 ms/cm. 其中, 1.5 mol/L处理组下降的比例最高.8 d后各实验组的 电导率均开始上升,18 d后,分别高出其初始值 $0.07 \, \text{ms/cm} \, 0.03 \, \text{ms/cm} \, 0.85 \, \text{ms/cm} \, 4.12 \, \text{ms/cm}.$

培养基中pH值的变化与微藻的新陈代谢 活动密切相关. 当初始营养物质充足时, 微藻进 行光合作用吸收 CO2, 使培养基中 CO2的含量减 少,导致溶液中pH值升高(Liu et al., 2020),因 此在前4d培养基中pH值迅速升高.随着营 养物质、CO2的消耗殆尽,pH值维持稳定.此 外,由于受到不同盐胁迫的影响,微藻的生长 受到影响,吸收 CO2的能力受到不同程度的限 制,因此pH值的变化呈现一定的梯度效应.

电导率是测定溶液中溶解盐分含量的一个 重要指标(张小霓等,2004),对电导率的测定能 够反映溶液中盐离子的迁移和反应能力.培养基 中EC值在前8d整体呈下降趋势,主要是由于藻

类在培养初期生长迅速,藻细胞能够吸附和吸收盐离子.此外,pH的增加使部分盐离子发生沉淀,降低了盐离子的迁移能力和反应活性.组成细胞质的蛋白质是两性电解质,而氨基酸在弱碱性环境中带负电荷(郑云普等,2010),易于吸附外界溶液中的阳离子,在一定程度上促进微藻对Na⁺、Ca²⁺、Mg²⁺、K⁺等阳离子的络合.因此,EC值在前8d整体呈下降趋势.随着培养基中营养物质的消耗,部分藻体死亡,pH值趋于稳定,盐离子重新释放到环境中,导致电导率增加.

2.4 土壤微藻对培养基中盐分的影响

土壤微藻对培养基中盐分的影响如图 4 所示,18 d 后,实验组的可溶性全盐量均下降,其中下降量最大的为 1 mol/L 处理组,下降了 6.96 g/L.此外,0.5 mol/L 和 1.5 mol/L 的处理组分别下降 6.13 g/L,3.42 g/L,对照组基本没有变化.

微藻的耐盐性通过调控胞内离子稳态(K+和 Ca²⁺)、合成渗透调节物质(脯氨酸、甘油和甘氨酸甜 菜碱等)、改变膜结构、激活酶促和非酶促抗氧化防 御系统以及改变光合作用途径等生化和分子途径 实现(Singh et al., 2018). 盐胁迫下, Na⁺与 Ca²⁺竞 争,与细胞壁结合,一定程度上起到调控离子的作 用. 微藻在盐胁迫条件下,能够产生胞外聚合物与 盐离子发生反应,从而降低了培养基中盐分的含量. 与 0.5 mol/L 处理组相比,1 mol/L 处理组含盐量相 对较高,可能会刺激藻类产生更多的胞外聚合物, 因此 1 mol/L 处理组的可溶性全盐量下降量高于 0.5 mol/L. 然而,高盐度条件下由于 Na+的含量较 高,打破了细胞离子内稳态,导致K⁺水平降低,细胞 壁收缩,影响细胞生长(Demidchik et al., 2014),从 而降低了对盐分的去除,因此1.5 mol/L处理组 的可溶性全盐量下降量低于1 mol/L 处理组.

2.5 土壤中微藻的生长

土壤环境中微藻生物量的变化如图 5 所示,在接种微藻后,前 5 d,各处理组藻类生物量生长缓慢,随后持续上升到第 10 d. 第 10~15 d 内,不仅 3 μ g/cm² 和 8 μ g/cm² 土壤处理组的生长下降,5 μ g/cm² 土壤处理组也呈现下降 .15 d后3 μ g/cm² 和 8 μ g/cm² 土壤处理组生物量迅速增长 . 培植 20 d后,初始叶绿素 a 接种量为 3 μ g/cm²、5 μ g/cm²、8 μ g/cm² 土壤处理组的生物量分别增加 4.99 μ g/cm²、3.4 μ g/cm²、8.54 μ g/cm² 土壤,表明土壤微藻可以在盐碱地中生长 .

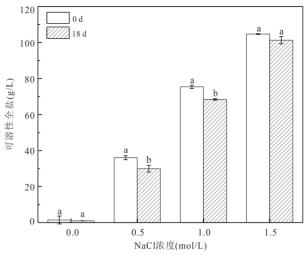


图 4 微藻对液态环境中可溶性全盐的影响

Fig. 4 Effect of microalgae on soluble total salt in liquid environment

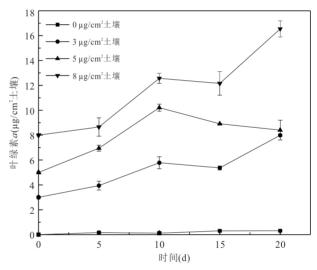


图 5 土壤中微藻生物量的变化

Fig. 5 Changes of microalgae biomass in soil

2.6 微藻对土壤 pH和EC的影响

接种微藻后土壤 pH和EC的变化如图 6 所示.由图 6a可知,土壤初始 pH值为 9.37,为碱性土壤,接种土壤微藻后,前 5 d内 pH缓慢上升,最大值为 9.50,随后有所下降,但总体变化不明显,可见,接种微藻后,经过 20 d的培植,土壤 pH并无明显改变.由图 6b可知,土壤藻接种 5 d内,各实验组的土壤电导率均有轻微的降低;第 5~10 d内,各组电导率迅速升高.第 10 d后,各实验组均处于下降趋势,只有对照组和 3 μg/cm²土壤处理组在第 15~20 d内有所升高.但整体而言,20 d后,各实验组的土壤电导率只有较小幅度增加.

土壤pH值直接影响植物的生长和微生物活性

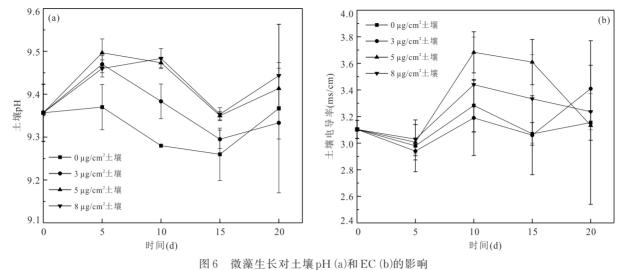


图 0 版採生区州土泰 pri (a//fi EC (b//fi) 彩·阿

Fig. 6 $\,\,$ Effects of microalgae growth on soil pH (a) and EC (b)

以及土壤的其他理化性质.土壤pH在初期升高,主要是由于土壤微藻生长时利用土壤中营养物质,导致土壤pH升高.随着部分藻体的死亡,藻类分解产生的有机物可能一定程度降低了土壤pH值,导致后期pH值下降.此外,土壤电导率作为衡量土壤盐渍化程度的重要指标之一,微藻在接种初期,土壤电导率轻微下降,可能是微藻生长时吸收利用了土壤中的部分盐离子.但随着微藻的生长,其分泌的代谢产物,如小分子有机酸(吲哚乙酸,琥珀酸等)促进土壤颗粒中矿质离子释放,从而使电导率升高.

2.7 土壤含水率的变化

图 7 为土壤含水率的变化情况. 经过 20 d 的培养,接种叶绿素 a 为 3 μg/cm²,5 μg/cm²,8 μg/cm² 土壤的实验组含水量分别增加了 29.41%,38.29%,39.54%. 不同初始接种量的实验组其含水率不同,其中高接种量具有相对较高的含水率. 因此,土壤微藻具有提高土壤保水能力的作用.

由于新疆地区土壤类型主要为荒漠土,土壤质地以细沙和粉沙为主,蒸发量大,土壤的保水能力差(Wang et al., 2018).一些丝状蓝藻的细胞外具有较厚的黏胶层或胶鞘,能够大量吸收雨水或露水(谢作明等,2007),不仅为藻细胞营造湿润的微环境,而且能起到土壤保水的作用.接种土壤微藻,加速了表面生物结皮的快速形成,减少了土壤水分的蒸发,进一步能阻止盐分运移.接种量的不同能够使土壤保水能力具有差异性.此外,土壤含水越高稀释盐分的作用越明显,从而减轻盐分对作物及土壤微生物的胁迫,对快速构建良好、健康的土壤环境起到辅助作用.

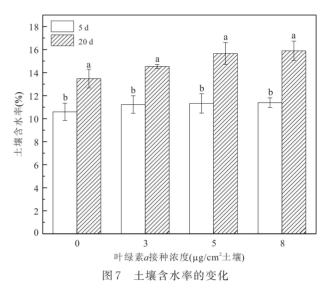


Fig. 7 Changes of soil moisture content

2.8 盐胁迫对土壤中微藻分泌 EPS 的影响

不同微藻接种量条件下土壤中 EPS 的变化如图 8 所示 . 经过 20 d 的培养 ,接种量为 3 μ g/cm² ,5 μ g/cm² 和 8 μ g/cm² 土壤处理组中 EPS 的含量均增加 ,分别增加了 82.84% ,86.04% 和 116.06% .EPS 含量增加率与微藻接种量成正相关 .

土壤藻在盐碱土中通过分泌 EPS来减轻盐胁 迫对其生长的影响.EPS中的部分官能团,如-OH、-COOH、-NH和-C=O(De Philippis et al., 2003)络 合盐离子(如,Na⁺,Mg²⁺、Ca²⁺)(Arp et al., 1999; Mishra et al., 2011),维持细胞内外环境的渗透压平 衡,减轻盐离子对藻细胞的伤害.分泌的 EPS也可 作为碳源被藻细胞重新吸收,藻细胞对有机物和盐 离子的选择性利用,提高了微藻的耐盐性(Chen et

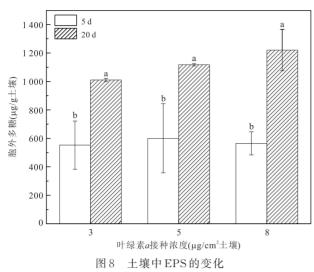


Fig. 8 Changes of EPS in soil

al.,2003). 微藻的 EPS 分泌量与其生物量和 光合作用强度有关(陈兰洲等,2002). 随着土壤中微藻生物量和 EPS 的增加,土壤团聚体增加,进一步限制土壤中盐离子的迁移.

3 结论

- (1)从新疆盐渍化土壤表层生物结皮中筛选的一株微藻,能够在1 mol/L NaCl溶液中存活并生长,具有较高的耐盐性.
- (2)盐胁迫降低了微藻光合色素的含量,表现出明显的梯度效应.微藻能够降低溶液中可溶性盐含量,其中,对低浓度可溶性盐的去除效果相对较好.
- (3)微藻在盐碱土表层生长时,增加了土壤含水率,提高了土壤保水能力,增加了土壤中EPS含量,一定程度能够限制土壤中盐分的迁移.

因此,接种土壤微藻可以改善土壤理化性质, 改良盐渍化土壤,从而提高土壤可利用性,为盐渍 化土壤治理和生态修复提供新的途径和理论依据.

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